



SHORT COMMUNICATION

Senegal Flapshell Turtle (*Cyclanorbis senegalensis*) in Ethiopia (Testudines: Trionychidae)

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Abstract.—Based on DNA sequences derived from two hatchlings from the Alwero river, Gambela Region, *Cyclanorbis senegalensis* is recorded for the first time for western Ethiopia. Previously published DNA sequences of *C. senegalensis* from Benin and Togo are slightly different, suggesting phylogeographic structure.

Keywords. Africa, Alwero river, Cyclanorbinae, first record, Gambela Region, Reptilia

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Flapshell turtles (Cyclanorbinae) are a subfamily of softshell turtles (Trionychidae) that include four species in two genera (*Cycloderma*, *Cyclanorbis*, each with two species) in sub-Saharan Africa, and three species of the genus *Lissemys* in the Indian subcontinent, Myanmar, and perhaps Thailand (Branch 2008; Praschag et al. 2011; van Dijk et al. 2014). The Asian species constitute the sister group to the African taxa (Engstrom et al. 2004). All cyclanorbines are characterized by the presence of femoral and caudal flaps on the plastron, together with the movable plastral forelobe allowing the complete closure of the shell—a unique character among softshell turtles.

The African species have been relatively little studied and many records are historical (Gramentz 2008; Broadley and Sachsse 2011; Baker et al. 2015; cf. also the EMYSsystem Database). The two *Cyclanorbis* species, *C. elegans* and *C. senegalensis*, co-occur throughout much of their distribution (Fig. 1), with *C. senegalensis* having a much wider range. However, for non-specialists, the two species are not easy to tell apart. They differ in adult size and *C. elegans* has two, or less commonly, four plastral callosities, while the smaller *C. senegalensis* has up to nine callosities (Branch 2008; Baker et al. 2015). Yet, considering that the number of plastral callosities may change during growth, it is obvious that

the two species can be easily confused and that some records of either species may actually refer to the other. In particular hatchlings and small juveniles are difficult to determine.

Even though Ethiopia is included in the distribution ranges of *C. elegans* and *C. senegalensis* by the IUCN Red List of Threatened Species, neither species has ever been recorded from that country (Largen and Spawls 2010). However, the two species are known from the White Nile system close to the Ethiopian border (EMYSsystem Database). Largen and Spawls (2010) suggested that both *C. elegans* and *C. senegalensis* may occur in the Baro river in Ethiopia because records exist downstream in South Sudan, less than 30 km from the border. Also Baker et al. (2015) speculated that *C. elegans* could occur in westernmost Ethiopia, corresponding to the catchment basins of the Baro and Akobo rivers, both tributaries of the White Nile.

On 26 August 2014, during an excursion to Ethiopia, one of us (V.T.) obtained two hatchlings of a flapshell turtle near Ugudi village at the Alwero river (Gambela Region, 7°58'55.83"N, 34°16'06.12"E, 439 meters above sea level.). The Alwero river is a tributary of the Baro river and the collection site is approximately 150 km distant from the closest known occurrence of the species in South Sudan (Nasir; Siebenrock 1909; Largen

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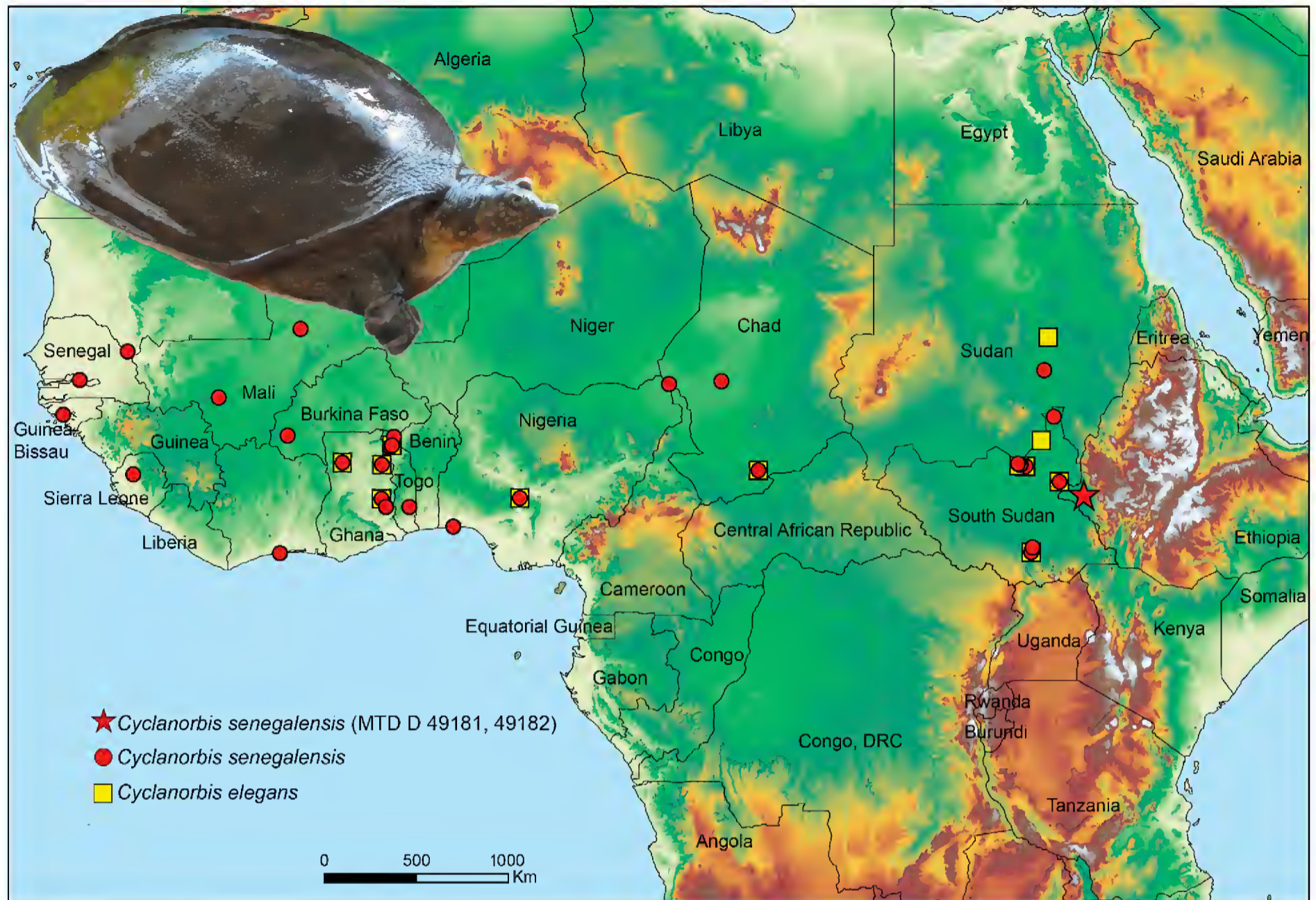


Fig. 1. Distribution of *Cyclanorbis elegans* and *C. senegalensis* (localities from EMYSsystem Database plus additional records from Sierra Leone, see inset, and Ethiopia). Inset: Adult *C. senegalensis* from Sierra Leone, Rokel river at Robung village. Photo: Bill Branch.

and Spawls 2010). The two specimens (Fig. 2) are now in the collection of the Museum of Zoology, Senckenberg Dresden (MTD D 49181, 49182). The turtles were caught by native children during daytime, in a temporary pool close to the river. The species is well-known to locals. In this region, late August is the climax of the rainy season and lush vegetation at the collection site comprised dense elephant grass. The straight line carapacial lengths of the two specimens were 47.9 mm (MTD D 49181) and 40.6 mm (MTD D 49182).

Tissue samples of the ethanol-preserved hatchlings were used for genetic characterization because species identity was unclear. Laboratory procedures and

phylogenetic calculations are described in detail in the Supporting Information of this article (Dryad Repository, <http://dx.doi.org/10.5061/dryad.g48pb>). Sequences of three mitochondrial DNA blocks (in total 2,429 bp) that had previously been shown to be taxonomically informative for softshell turtles (e.g., Engstrom et al. 2004; Fritz et al. 2010, 2014; Praschag et al. 2011) were generated. Fragment 1 corresponded to 398 bp of the 12S rRNA gene. The 1,140-bp-long fragment 2 represented the complete cytochrome *b* (*cyt b*) gene. Fragment 3 contained 709 bp of the NADH dehydrogenase subunit 4 gene (ND4) and 182 bp of adjacent DNA coding for tRNAs. These individual DNA

Species	Provenance	Reference	12S	<i>cyt b</i>	ND4+tRNAs
<i>Cyclanorbis elegans</i>	Benin	Engstrom et al. (2004)	n/a	AY259570	AY259615
<i>Cyclanorbis senegalensis</i>	Benin	Praschag et al. (2011)	FR850553	FR850654	FR850604
<i>Cyclanorbis senegalensis</i>	Togo	Engstrom et al. (2004)	n/a	AY259569	AY259614
<i>Cyclanorbis senegalensis</i>	Ethiopia: Ugudi village, Gambela Region	This study (MTD D 49181)	LT595717	LT595719	LT595722
<i>Cyclanorbis senegalensis</i>	Ethiopia: Ugudi village, Gambela Region	This study (MTD D 49182)	LT595718	LT595720	LT595723
<i>Cycloderma aubryi</i>	Congo Brazzaville: Pointe Noire	Praschag et al. (2011)	FR850554	FR850655	FR850605
<i>Cycloderma aubryi</i>	Congo Brazzaville: Tchingoli	Praschag et al. (2011)	FR850555	FR850656	FR850606
<i>Cycloderma aubryi</i>	Gabon	Engstrom et al. (2004)	n/a	AY259566	AY259611
<i>Cycloderma frenatum</i>	Lake Malawi	Engstrom et al. (2004)	n/a	AY259565	AY259610
<i>Lissemys ceylonensis</i>	Sri Lanka: Colombo	Praschag et al. (2011)	FR850544	FR850645	FR850595

Table 1. Used samples and GenBank sequences and their accession numbers.

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Fig. 2. Hatchlings of *Cyclanorbis senegalensis* from the vicinity of Ugudi village at the Alwero river, Gambela Region, western Ethiopia. Left: MTD D 49181, right: MTD D 49182. Scale bars, 1 cm.

blocks were concatenated and merged for calculations with previously published homologous data of all four African flapshell turtle species. Sequences of *Lissemys ceylonensis* were included as outgroup (Table 1). Phylogenetic trees were then computed using Bayesian Inference and Maximum Likelihood approaches as implemented in MRBAYES 3.2.1 (Ronquist et al. 2012) and RAxML 7.2.8 (Stamatakis 2006). In addition, uncorrected *p* distances between concatenated DNA sequences were

calculated using MEGA 5.05 (Tamura et al. 2011) and the pairwise deletion option.

Both tree building methods yielded identical topologies (Fig. 3), with the western Ethiopian samples clustering with maximum support with *C. senegalensis*. However, while the sequences of the Ethiopian samples were completely identical, they differed slightly from sequences for *C. senegalensis* from Benin and Togo. When uncorrected *p* distances were compared, sequence

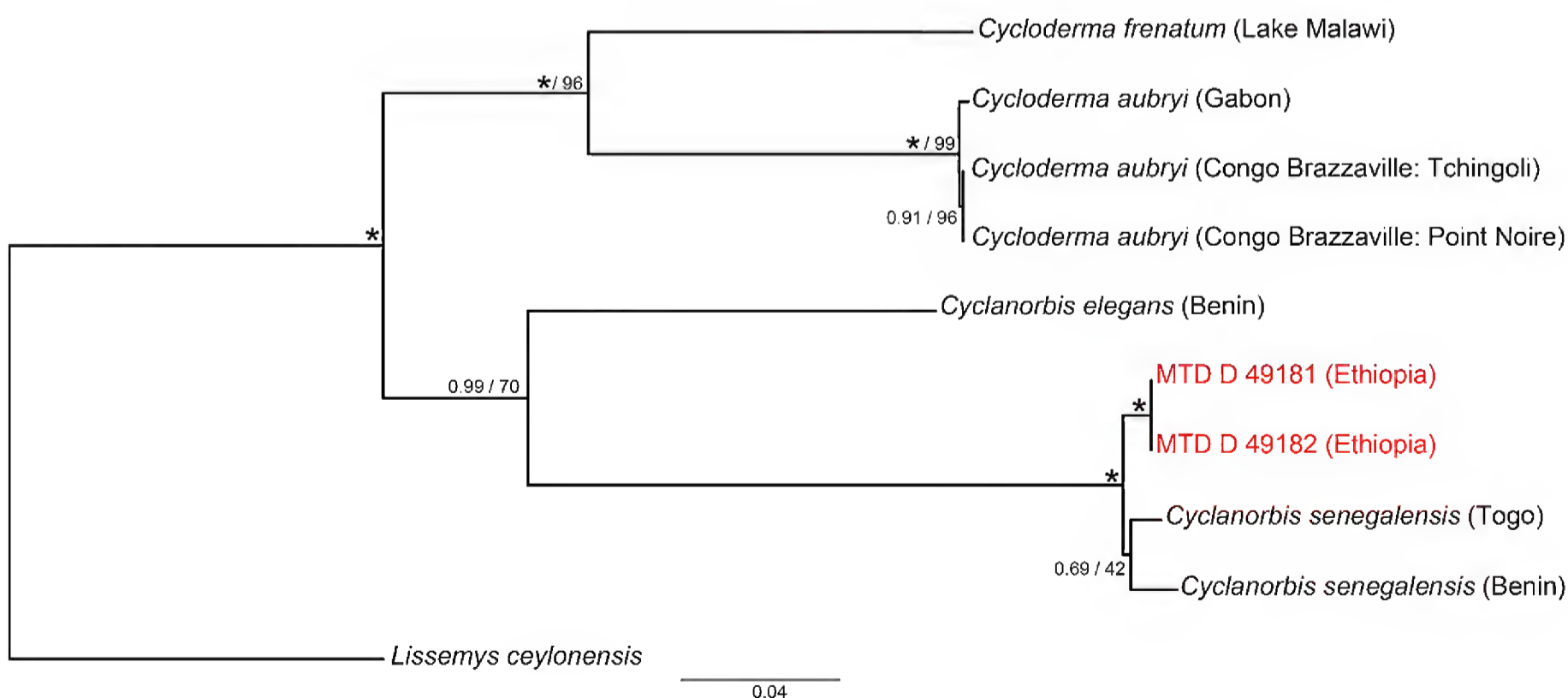


Fig. 3. Maximum Likelihood (ML) tree for flapshell turtles (Cyclanorbinae) using 2,429 bp of mtDNA. Values at nodes are Bayesian posterior probabilities and ML bootstrap support. Asterisks indicate maximum support under one or both approaches. Note placement of Ethiopian samples (in red).

divergences among the Ethiopian samples and the *C. senegalensis* from Benin and Togo ranged between 1.5% and 1.9%, whereas divergences between *C. elegans* and *C. senegalensis* differed by one order of magnitude (15.2–15.4%; cf. Supporting Information). Thus, the hatchlings from western Ethiopia are clearly referable to *C. senegalensis*. The observed sequence divergences within *C. senegalensis* suggest that phylogeographic structuring exists and that further studies should be conducted to examine this situation.

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Tomáš Mazuch is a Czech amateur herpetologist. Since his teenage age he has dedicated his life to the breeding of amphibians, reptiles, and invertebrates. During his studies of Veterinary Sciences (not finished yet) he began to devote his research to herpetology and parasitology of reptiles. His research focuses on taxonomy, systematics, and biogeography of amphibians and reptiles of the Horn of Africa (mainly Somalia and Ethiopia). His main subjects of study are geckos of the genus *Hemidactylus* from Eastern Africa. He is also interested in the taxonomy of scorpions from North-Eastern Africa. He has authored or co-authored over 15 peer-reviewed papers and books on parasitology, systematics of scorpions, and herpetology, including the book “Amphibians and Reptiles of Somaliland and Eastern Ethiopia, ...based on two field trips in 2010/2011” (2013). He has co-authored the descriptions of six reptile and four scorpion species.



Vladimír Trailin was born in Susice, a town in southern Bohemia in the Šumava Mountains. The neighbourhood of the Šumava National Park formed his interest of nature. In 1978–1982, he graduated from the High School of Mechanical Engineering, but remained continuously interested in nature and natural history, especially entomology and herpetology. He travelled many times to the Balkan Peninsula, also to Cuba, and for the first time to Africa in 1992. Since then he has repeatedly visited Ethiopia, and together with colleagues has rediscovered some poorly known snakes (e.g., *Lamprophis erlangeri* and *Bitis parviocula*), as well as the first live individuals of *Pseudoboodon boehmei*. The species *Cicindela trailini* (Coleoptera), *Hottentotta trailini* (Scorpiones), and *Pandinus trailini* (Scorpiones) are dedicated to him.



Uwe Fritz is director of the Senckenberg Natural History Collections at Dresden, Germany, and extraordinary professor for zoology at the University of Leipzig. He has worked for many years on the taxonomy, systematics, and phylogeography of turtles and tortoises, and also studied to a lesser extent snakes and lizards. He is particularly interested in hybridization patterns and gene flow in contact zones of distinct taxa. Uwe has authored or co-authored numerous scientific articles, mainly in herpetology, and has also edited proceedings and books, among them the two turtle volumes of the “Handbook of Amphibians and Reptiles of Europe.” He is currently the President of the German Society for Biological Systematics, and is responsible for the Senckenberg collections and the scientific journals edited by the Senckenberg Society.



Melita Vamberger is a Slovenian herpetologist and evolutionary biologist. She studied biology at the University Ljubljana, with the focus on the natural history of the European pond turtle (*Emys orbicularis*). After her diploma she moved to Germany and studied for her Ph.D. thesis (2014) at the University of Leipzig on the phylogeography and hybridization of two closely related freshwater turtles (*Mauremys caspica* and *M. rivulata*). Since then she has been employed as a researcher at Senckenberg Dresden. Her main interests are studying processes of speciation, gene flow, and evolution in different turtle taxa using genetic methods, with a focus on the Western Palearctic and sub-Saharan Africa.